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Structure–activity relationships of pyrithiones – IPC-81 toxicity tests with the antifouling biocide zinc pyrithione and structural analogs

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Zinc pyrithione (1-hydroxypyridine-2-thione, zinc complex; $ZnPT_2$) is currently viewed as the top prospect for replacing tributyltin antifoulants in ship paints. Thus, the risk assessment of a high scale release of $ZnPT_2$ to the natural environment is of increasing importance. The knowledge of the molecular mechanisms related to biological effects of $ZnPT_2$ and its transformation products is crucial for this assessment and thus for the decision whether pyrithiones are sound or "green" alternatives to organotin antifoulants. A multitude of biological effects of pyrithiones is already known while the underlying molecular mechanisms of action remain obscure. This study presents toxicological data of zinc pyrithione and several structural analogs in rat leukemic cells (IPC-81). The N-hydroxythioamide functional group proved to play a significant role in the molecular mechanisms related to the biological action. Structural analogs, which are deprived of one or more molecular interaction or chemical reaction potentials given by this group (namely pyridine, pyridine 1-oxide and pyridine 2-thione, bis(2-pyridinyl)disulfide, and three methylated metabolites), exhibit far less toxic potential in IPC-81 cells than pyrithiones (*i.e.*, 2-pyridinethione-1-oxides). In particular the trans-metallization products of $ZnPT_2$, iron ($FePT_3$) and copper ($CuPT_2$) pyrithione, and the oxidation product bis(2-pyridinyl)disulfide 1,1′-dioxide (pyrithione disulfide, (PT_2)) have been proven to be as toxic as $ZnPT_2$ and tributyltin chloride in IPC-81 cells. $CuPT_2$, $FePT_3$ and $(PT)_2$ need to be considered as environmental transformations products of $ZnPT_2$.

1 Introduction

1-Hydroxy-2-pyridinethione **1**, known as Pyrithione (PT) or Omadine®, is an aromatic heterocycle related to pyridine. *Via* the sulfur and the oxygen of its N-hydroxythioamide group, it forms complexes with most transition metals.[1–3] For fifty years PT has been noted for its highly bacteriocidal and fungicidal action.[4–6] Often metallization of the bidentate ligand highly augments biocidal action as in the case of zinc pyrithione **2** (Zinc Omadine®, $ZnPT_2$). (Fig. 1).

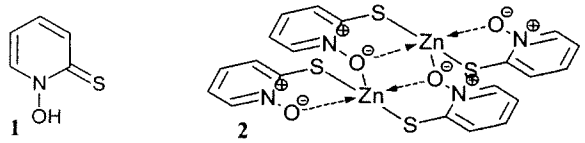

Fig. 1 Pyrithione **1** and zinc pyrithione **2**. In the solid state $ZnPT_2$ exhibits a dimeric structure with pentacoordinated zinc.[10]

$ZnPT_2$, the derivative of PT that is produced prevalently, is a well-known chemical. It is employed as a preservative in various commercial products such as cosmetics or industrial fluids. It is also an effective antidandruff agent, and it has been used in several hair care products for the last 30 years (*e.g.*, Head & Shoulders®, Dove®). Pyrithione also exhibits a broad action against marine fouling organisms and thus finds application as a booster biocide in anti-fouling paints.[7–9]

Due to the ban of organotin antifoulants in ship paints established by the International Maritime Organization (IMO), the use of substitute biocides is of increasing significance. For example, Arch Chemicals, producers of $ZnPT_2$ have announced substantial production growth for 2002 and expect to become leaders in the antifouling biocide market by 2003 or 2004.[11]

Biofouling on ship hulls is caused by a large diversity of aquatic organisms (*e.g.*, different algae, mussels or barnacles). High toxicity against a broad spectrum of different organisms is a feature of antifouling biocides desired by the user. Thus, it is not surprising that pyrithiones have been found to induce a large variety of toxic effects in highly diversified living organisms, which in most cases are non-target organisms.

Pyrithiones have been found to produce teratogenic and general toxic effects in aquatic organisms (fish and sea urchins) at extremely low concentrations†.[12,13] In a comparative investigation using sea urchin eggs, toxicity of pyrithiones is far higher than that induced by tributyltin oxide.[13] Genotoxic effects induced by pyrithiones have been found in mammalian lymphoma cells with and without photoactivation.[14] Pyrithiones are general inhibitors of membrane transport processes in fungi[15] and have been found to distort membrane integrity in bacteria[16–19] and mammalian cultured cell lines.[20] Oxidative damage elicited by pyrithiones has been found in rabbit myocardium tissue.[21] Membrane depolarization induced by pyrithiones has been shown in mammalian cultured cells[22] and fungi.[23] Pyrithione biocides are capable of interaction with common biological molecules (phosphatidyl-ethanolamine, cysteine)[16] and have been shown to cause large decreases in ATP levels in bacteria[18] and fungi.[15] As ionophores, pyrithiones affect homeostasis of different metal cations. This was found in different mammalian cultured cell lines[22,24,25] and rabbits (*in vivo*).[26] Further, pyrithiones affect amino acid metabolism in different mammalian cultured cell lines[25,27,28] as well as nucleoside

† Early life stage tests exhibited significant teratogenic effects (morphologically visible wavy structures of the vertebral column) at very low concentrations of $ZnPT_2$ in larvae of zebra fish and Japanese Medaka.[12] $ZnPT_2$ exhibited EC_{50} values of 9 μg L^{-1} [28 nM] in zebra fish and 5 μg L^{-1} [16 nM] in Japanese Medaka. Kobayashi and Okamura[13] comparatively assessed the effects of tributyltin oxide and seven organotin antifoulant substitutes on sea urchin eggs and embryos. With No Observed Effect Concentrations (NOEC) of 0.03 attoM, $ZnPT_2$ was the most toxic antifoulant tested. $CuPT_2$ exhibited a NOEC value of 3 femtoM.

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metabolism in bacteria,[29] different mammalian cultured cell lines[27,28,30] and the isolated salmon sperm DNA/bacterial RNA polymerase model.[29] Pyrithiones affect cellular function regulating molecules (transcription factors NF-κB and AP-1) in different mammalian cultured cells.[24]

Numerous biological effects of pyrithiones are known but the identity of the active species and the molecular causalities of this highly diverse biological activity remain obscure. However, this knowledge is crucial for the assessment of the risk implied by a high scale release of pyrithione biocides to the natural environment, and it is thus important for the decision whether pyrithiones biocides are sustainable alternatives to tributyltin antifoulants.

The present study was aimed at the comparative evaluation of the toxic potentials of $ZnPT_2$ and some structural analogs. Copper pyrithione **3** ($CuPT_2$), iron pyrithione **4** ($FePT_3$) and bis(2-pyridinyl)disulfide ($(PT)_2$) **5** have been selected because they are probable environmental transformation products of $ZnPT_2$, as will be discussed later. Free pyrithione **1** is prone to oxidation and therefore relatively difficult to handle. Thus, sodium pyrithione **6** (NaPT) has been selected to represent the free pyrithione **1** or pyrithionate **1a**. At the low concentractions at which NaPT is active the salt might completely dissociate with the PT moiety entering the acid–base equilibrium according to its pK_a of 4.6 (Fig. 4 and Table 3). Pyridine **7** (Py), pyridine 2-thione **8** (PyS), and pyridine 1-oxide **9** (PyNO) are the closest structural analogs of PT. Bis(2-pyridinyl)disulfide **10** ($(PyS)_2$) is a close analog of $(PT)_2$. Three methylated metabolites of $ZnPT_2$ which have been found in rats[6] have been selected: 2-(methylthio)pyridine 1-oxide **11** (MSPT), 2-(methylthio)pyridine **12** (MSP) and 2-(methyl-sulfonyl)pyridine **13** (MSO_2P). Methylated metabolites are also likely to be part of metabolic pathways of bacteria. For comparison, EC_{50} values of tributyltin chloride **14** (TBT) are given. The substances tested are listed in Table 1.

Toxicities of these "testkit-compounds" were determined with the WST/IPC-81 cell viability assay. This assay has been successfully performed in this laboratory before for hazard assessment and mode of action studies.[31-33] IPC-81 is a cultured promyelocytic leukemia rat cell line,[34] WST-1 reagent is a dye which is electrochemically reduced by living cells. This reduction is seen as changing absorbance (450 nm), which in turn is used as an indicator for cell viability.

The interpretation and discussion of the results has been performed according to the "T-SAR" approach[35] – a bioorganic chemistry approach of "thinking in terms of structure–activity and structure–property relationships". This approach is aimed at the systematic comprehension of the molecular structures of chemicals focusing on features such as chemical reaction and noncovalent interaction potentials, functional groups, stereochemistry and possible transformation or speciation of these chemicals as well as on possible impacts of identified characteristics in biological systems. A systematic characterization of $ZnPT_2$, PT and NaPT according to T-SAR is given in ref. 36 This includes a comprehensive review and classification of observed biological effects of pyrithiones.

2 Experimental

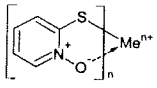
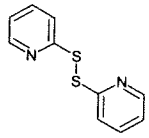
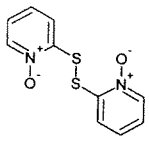
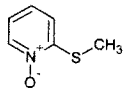
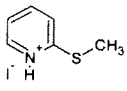
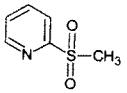
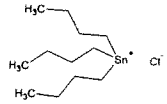
(a) Chemicals

Testkit compounds. All chemicals that were tested for toxicity, except for PyNO and Py, had been packed in 1 and 0.1 μmol portions. This had been done by measuring respective aliquots from a stock solution followed by lyophilization. PyNO and Py were directly applied from stock solutions.

Pyridine **7** of analytical grade was obtained from Bernd Kraft GmbH, pyridine 1-oxide **9** and pyridine 2-thione **8** (97%) from Fluka. Zinc pyrithione **2** 95% and sodium pyrithione **6** 97% were purchased from Sigma. Bis(2-pyridinyl)disulfide 1,1′-dioxide **5** 99% was synthesized as described in ref. 37 and characterized by UV and ESI-MS. Purity of the compounds was assessed using HPLC. Bis(2-pyridinyl)disulfide **10** (97%) was obtained from Janssen. Tributyltin chloride 96% **14** was purchased from Aldrich.

Copper and iron pyrithione **3** and **4** were obtained by drop-wise addition of an excess of aqueous solutions of either cupric nitrate

Table 1 Structural analogs selected for IPC-81 toxicity tests

Molecular structure	Chemical name	Abbr.	No.	Molecular structure	Chemical name	Abbr.	No.
 Me = Zn^{2+}, Cu^{2+}, Fe^{3+}	Zinc(II)- Copper(II)- Iron(III)- pyrithione	$ZnPT_2$ $CuPT_2$ $FePT_3$	**2** **3** **4**		Pyridine 1-oxide	PyNO	**9**
					Bis(2-pyridinyl)disulfide	$(PyS)_2$	**10**
	Bis(2-pyridinyl) disulfide 1,1′- dioxide	$(PT)_2$	**5**		2-(Methylthio) pyridine 1-oxide	MSPT	**11**
	Sodium pyrithione	NaPT	**6**		2-(Methylthio) pyridine hydroiodide	MSPHI	**12**
	Pyridine	Py	**7**		2-(Methylsulfonyl) pyridine	MSO_2P	**13**
	Pyridine 2-thione	PyS	**8**		Tributyltin chloride	TBT	**14**

trihydrate or ferric chloride hexahydrate, respectively, (obtained from Fluka puriss. p.a. grade) to a stirred aqueous solution of sodium pyrithione. The metal complex precipitated immediately. To complete the reaction, the stirred mixture was heated to reflux. After cooling to room temperature, the mixture was filtrated. The precipitate was washed with water, and then with small amounts of methanol before lyophilization. The complexes were recrystallized from methanol and characterized by ESI-MS^n, UV/Vis, and in the case of $FePT_3$ by X-ray diffraction.

2-(Methylthio)pyridine 1-oxide **11** was obtained by stepwise addition of methyl iodide (99%, Acros Organics) to an equimolar amount of sodium pyrithione which was dissolved in 250 mL ethanol (room temperature). The mixture was left for 12 hours before the filtrate was separated from a white precipitate. The filtrate was evaporated and the beige precipitate recrystallized from methanol. Faint yellow crystals have been characterized by ^1H NMR and ESI-MS^n. Purity of the compounds was assessed using HPLC.

2-(Methylthio)pyridine **12a** was obtained by stepwise addition of methyl iodide to an equimolar amount of pyridine 2-thione which was dissolved in 300 mL ethanol (room temperature). The mixture was left for 12 hours before precipitated faint yellow crystals were separated from the filtrate. Crystals were washed with small amounts of cold ethanol and then recrystallized from ethanol. Faint yellow crystals of 2-(methylthio)pyridine hydroiodide **12** have been characterized by ^1H NMR and ESI-MS^n. Purity has been controlled by HPLC. Attempts failed to completely dry and exactly portion the free base, which was obtained by addition of an aqueous solution of sodium hydroxide to an aqueous solution of **12**. Therefore, **12** was used for toxicity tests accompanied by control experiments with sodium iodide, which exhibited no toxic effect.

2-(Methylsulfonyl)pyridine **13** has been obtained by stepwise addition of an excess of 30% H_2O_2 (Acros Organics) to a solution of 2-(methylthio)pyridine hydroiodide **12** which was dissolved in diluted acetic acid (Fluka) and heated to reflux. During addition the solution was permanently stirred. After the mixture was cooled to room temperature it was treated with concentrated sodium hydroxide (Fluka) and then extracted with *tert*-butylmethyl ether (Acros Organics). The clear colorless oil has been characterized by ESI-MS^n. Purity of the compounds was assessed using HPLC.

Chemicals for the WST/IPC-81 assay. Cell culture medium, serum and phosphate buffer were purchased from GIBCO BRL Life technologies, antibiotics and glutamine were obtained from PAA Laboratories and WST-1 Reagent (2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt) was purchased from Roche Diagnostics.

(b) The WST/IPC-81 cell viability assay

Cultures of IPC-81 were grown in RPMI 16/40 medium (Roswell Park Memorial Institute 160 Medium[38,39]) (supplemented with 0.2% $NaHCO_3$, 1% Penicillin/Streptomycin and 1% Glutamine) with 10% horse serum at 37 °C and a pH of 6.8 (5% CO_2).

In advance of the preparation of testkit stock solutions, compounds were suspended in DMSO to improve their solubilities. Final DMSO concentration in test media did not exceed 0.5%. Control experiments exhibited no toxic effects of DMSO in IPC-81 cells at this concentration. The stock solutions of the testkit compounds were prepared in culture medium and added to 96 well plates. Each plate contained three replicates of two substance dilution series, control and blank wells. Cells were added at a concentration of 15×10^5 cells mL^{-1} (in RPMI with 8% heat inactivated foetal calf serum) and cultivated for 48 hours. After 44 hours 10 μL of WST-1 reagent (diluted 1 : 4 with phosphate buffer) were added and cells were incubated for four hours. After incubation absorbance in each well (450 nm) was measured with a microplate-reader (MRX Dynatech). Cell viability was expressed as a percentage of absorbance compared to absorbance of controls.

1 : 1 dilution series generally started from 1 mM concentrations. If necessary, additional tests were performed with higher or lower dilution, respectively. Each dose–response curve was recorded for 3–6 repetitions (9–18 replicates).

(c) Effect data evaluation

The obtained data were normalised using blanks with no cells (mean response $\rightarrow$ 0) and unexposed cells (mean response $\rightarrow$ 1) on each microtiter plate. Dose–response curves were fitted to normalised data using the cumulative density function of a lognormal distribution. Wherever suitable, *i.e.* when the mean response at the highest concentration was lower than 0.5, EC_{50} values were calculated from the location parameter of the fitted density functions. EC_{50} values are only reported here if they did not exceed the highest concentration tested. Calculations were performed with the R language and environment for statistical computing, version 1.7, using the R package nls (nonlinear least squares).

3 Results

The viabilities with means and standard deviations as well as fitted dose–response curves are given in Fig. 2. For comparison all dose–response curves of the testkit compounds are shown together in Fig. 3. $\log_{10}EC_{50}$ values with standard deviations, respective EC_{50} values as well as the slopes *b* are given in Table 2.

Py **7**, PyNO **9**, MSPHI **12** and MSO_2P **13** exhibited no toxic effect in IPC-81 cells at concentrations below 1000 μM. EC_{50} values for PyS **8**, MSPT **11**, and $(PyS)_2$ **10** ranged from 60 to 480 μM. $ZnPT_2$ **2**, $CuPT_2$ **3**, $FePT_3$ **4**, NaPT **6** and $(PT)_2$ **5** exhibited very similar EC_{50} values ranging from 0.36–0.48 μM. TBT **14** exhibited an EC_{50} of 0.69 μM. $(PT)_2$ exhibited the lowest EC_{50} value of all pyrithione analogs tested.

For most substances the deviations in the dynamic range of the dose–response curves were relatively large. This might be due to the natural variability of the cells used combined with an "all-or-nothing" effect of the substances. This allows small concentration inaccuracies to cause relatively large deviations in viability. Orders of magnitudes and differences in the toxic potentials of the compounds tested are nevertheless evident. Differences in curve shapes will not be discussed in detail because of the high deviation in the dynamic range of the dose–response curves.

4 Discussion

The present results indicate two main structure–activity relationships of pyrithiones: (i) substances exhibiting the 2-thiopyridine-1-oxide structure ($ZnPT_2$, $CuPT_2$, $FePT_3$, NaPT and $(PT)_2$) exhibit far higher toxic potentials than those without this structure (Py, PyNO, PyS and $(PyS)_2$ as well as MSO_2P and MSPHI); (ii) the pyrithione complexes tested and sodium pyrithione exhibit similar and very low (nanomolar) effect concentrations. Both features can be explained with characteristics of the molecular structure of pyrithione considering physico-chemical data of pyrithione and its metal complexes.

Due to tautomerism and acid–base equilibria, pyrithione is subject to speciation in aqueous solution at physiological pH (Fig. 4). According to[40,41] the thione form is preferred by the factor 52 in water. The pK_a data obtained in the literature differ as shown in Table 3 and for calculations performed in this paper an average value of 4.6 has been used. According to Hendersson–Hasselbalch,

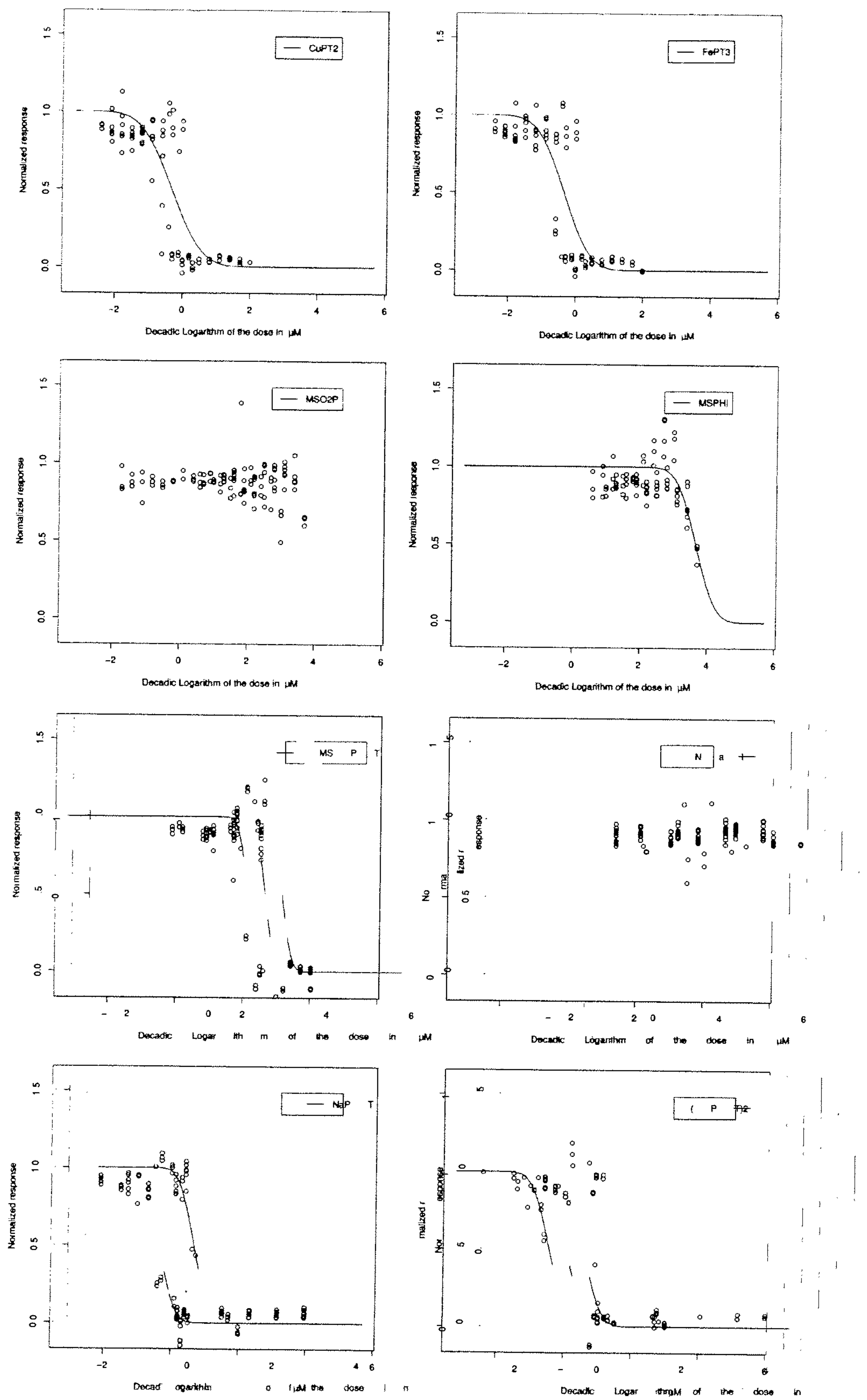


Fig. 2 Viabilities with means and estimated dose response curves for the compounds

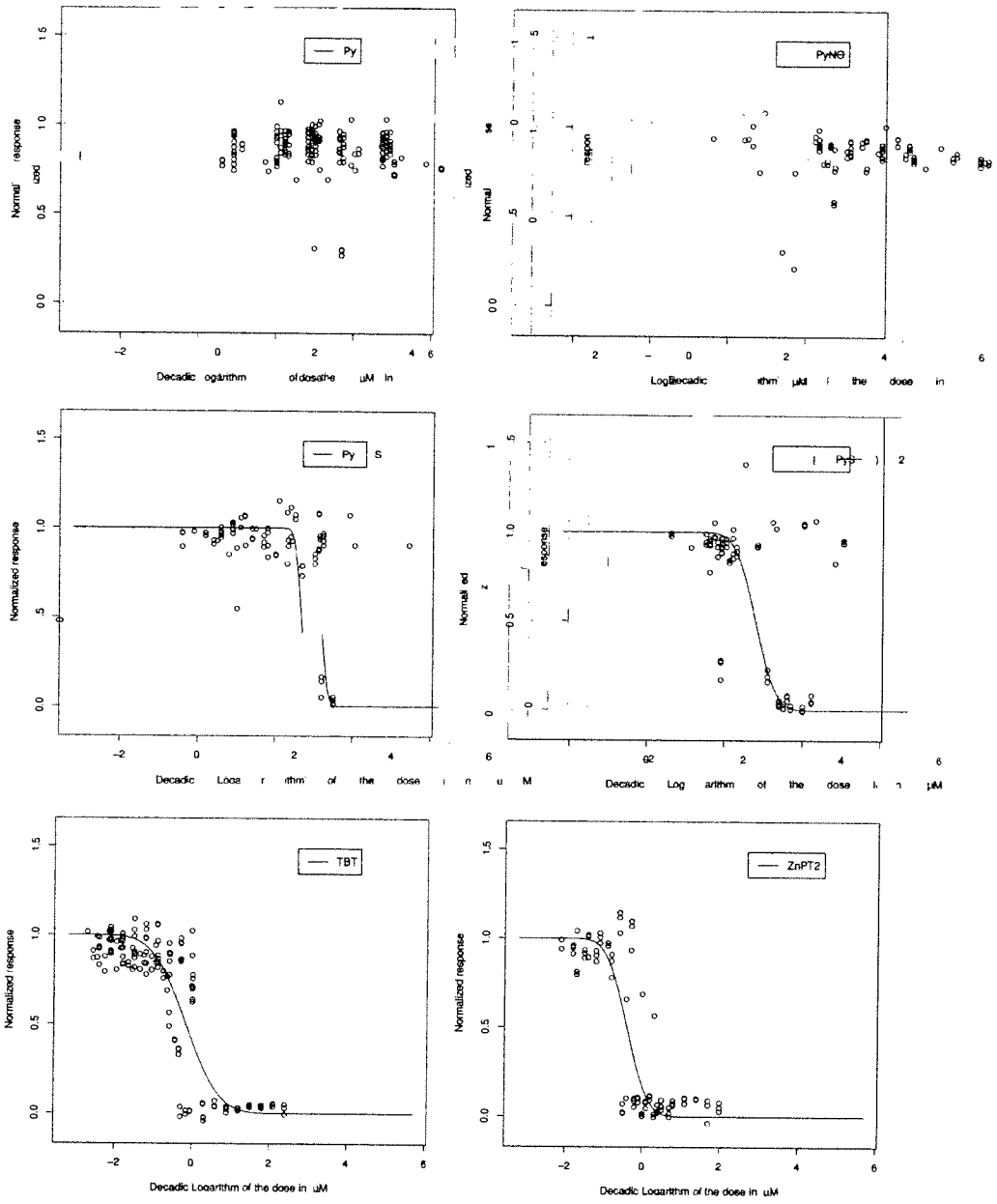


Fig. 2 cont.

at pH 7 deprotonated pyrithione exists in about 250-fold excess over the neutral form (pK_{a1} neglected).

The pyridine ring is capable of hydrophobic and π–π-interactions, whereas the N-hydroxythioamide group introduces highly polar properties to the molecule. The neutral molecule exhibits strong H-bond donation potential due to the exocyclic proton. The ability to enter a strong intramolecular H-bond may, however, hamper intermolecular H-bonds. Pyrithione exhibits H-bond acceptor potential due to several free electron pairs. The N-oxide group possesses a highly bi-polar structure. In the deprotonized forms of pyrithione, one negative ionic interaction potential is present. Pyrithione exhibits two potential nucleophilic sites, sulfur being a stronger nucleophile than the nitrogen-bound oxygen. Electrophilic potential, which is predominantly located at the nitrogen, may be hampered due to a missing leaving group.

According to eqn. 1 pyrithione is a reducing agent:

$$\text{R–SS–R} + 2e^- + 2H_3O^+ \rightleftarrows 2\text{R–SH} + 2H_2O \qquad E'_0 = -0.34‡ \quad (1)$$

Thus, free pyrithionate may generate $(PT)_2$ in the presence of mild oxidants as *e.g.* atmospheric oxygen or ferric ions. Oxidation of PT when exposed to air yielding $(PT)_2$ has been observed in our laboratory (Fig. 7).[36]

The chemical structure of the N-hydroxythioamide group in the pyrithionate anion species gives rise to a bidentate character due to the negative charge and the adjacent strong electron donor potential. This allows coordination to metal cations such as zinc(II), copper(II) or iron(III).

‡ Data obtained from[44] for PT *vs.* $Ag/AgCl/KCl_{sat}$ and from[43] for NaPT *vs.* SCE have been converted to E'_0 (SHE at pH 7) for the partial reaction $(PT)_2 + 2e^{\ominus} \rightarrow 2PT^{\ominus}$.

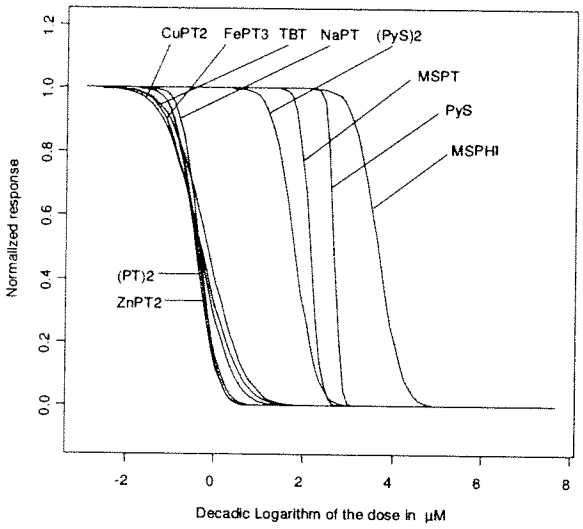


Fig. 3 Compiled dose–response curves for compounds tested.

Table 2 $\log_{10}EC_{50}$ values with standard deviations and respective EC_{50} values calculated from the dose–response curves of the testkit compounds in IPC-81 cells. Slopes b and number n of dilution series. All concentrations are given in [µM]

	$\log_{10}EC_{50}$	EC_{50}	n	b
$(PT)_2$	−0.44 ± 0.1	0.36	9	0.40
$ZnPT_2$	−0.40 ± 0.1	0.40	9	0.42
$FePT_3$	−0.35 ± 0.1	0.44	9	0.60
NaPT	−0.35 ± 0.07	0.45	12	0.34
CuPT2	−0.32 ± 0.2	0.48	9	0.69
TBT	−0.16 ± 0.1	0.69	15	0.68
(PyS)2	1.8 ± 0.1	60	9	0.41
MSPT	2.2 ± 0.07	140	12	0.21
PyS	2.7 ± 0.04	480	9	0.13
MSO_2P	≳ 3[a]	≳ 1000	12	
MSP(HI)	> 3	> 1000	9	
Py	> 3[a]	> 1000	18	
PyNO	> 3[a]	> 1000	9	

[a] No dose–response curve was fitted to the obtained data because the response was too low in the dose range measured.

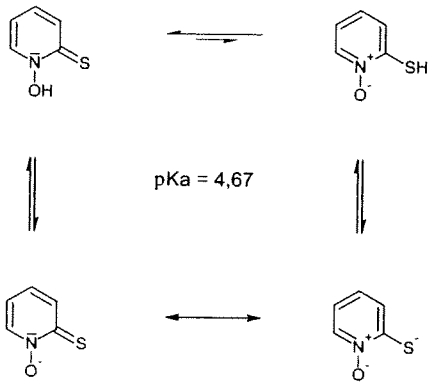


Fig. 4 Tautomerism and dissociation of PT.

In the presence of metal cations pyrithione is therefore subject to additional speciation as shown in Fig. 5 for $ZnPT_2$.

As shown in Fig. 6 and Table 4, at three calculated concentrations of applied $ZnPT_2$ ($ZnPT_{2(total)}$) pyrithionate is predominant.

Table 3 pK_a values of PT

	Method	
	UV spectral	polarographic
pK_{a2}	4.67 [40]	4.6 [43]
	4.40 [42]	
pK_{a1}	−1.95 [40]	

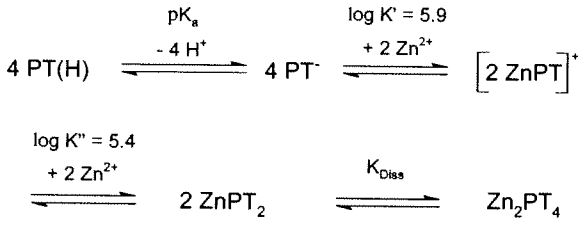


Fig. 5 Chemical equilibria of $ZnPT_2$.

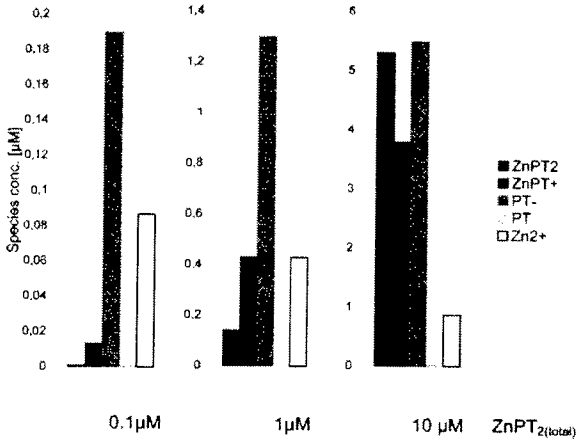


Fig. 6 Speciation of $ZnPT_2$ at different $ZnPT_2$(total) concentrations in aqueous solution at pH 7 resulting in various pyrithione species and free zinc(II). $ZnPT_2$ = zinc pyrithione 1 : 2, $ZnPT^+$ = zinc pyrithione 1 : 1, PT^- = pyrithionate, PT = free pyrithione, Zn^{2+} = free zinc(II) cations.

Table 4 Speciation of $ZnPT_2$. Different pyrithione species and free zinc(II) in aqueous solution of $ZnPT_2$(total) at pH 7. Concentrations are given in [µM]

$ZnPT_{2(total)}$	0.1	1	10
$ZnPT_2$	0.00059	0.14	5.3
$ZnPT^+$	0.013	0.43	3.8
$PT^\ominus$	0.19	1.3	5.5
PT	0.00074	0.0051	0.022
Zn^{2+}	0.087	0.43	0.87

Proportions of different pyrithione species and free zinc vary significantly at different $ZnPT_{2(total)}$ concentrations.§

Additional metal cations as *e.g.* Fe(III) or Cu(II) would split up the speciation even more according to the respective complex stability constants (Table 5). This speciation has been observed for PT, $ZnPT_2$ and $FePT_3$ in our laboratory (Fig. 7).[36] It also complicates the development of HPLC analysis methods for pyrithiones.

Each metal complex species of pyrithione exhibits characteristic molecular interaction potentials as *e.g.* positive ionic charge in the

§ Data have been calculated according to the Mass Law and the Henderson–Hasselbalch equation with the pK_a value of PT (Table 3) and stability constants of $ZnPT^+$ and $ZnPT_2$ (Table 5).

Table 5 Stability constants of pyrithione with zinc(II), iron(III) and copper(II)

	Zn^{2+}	Fe^{3+}	Cu^{2+}
$\log K'$	5.3 [1]	4.7 [2]	> 8.5 [2]
	5.9 [2]		
$\log K''$	5.4 [2]		

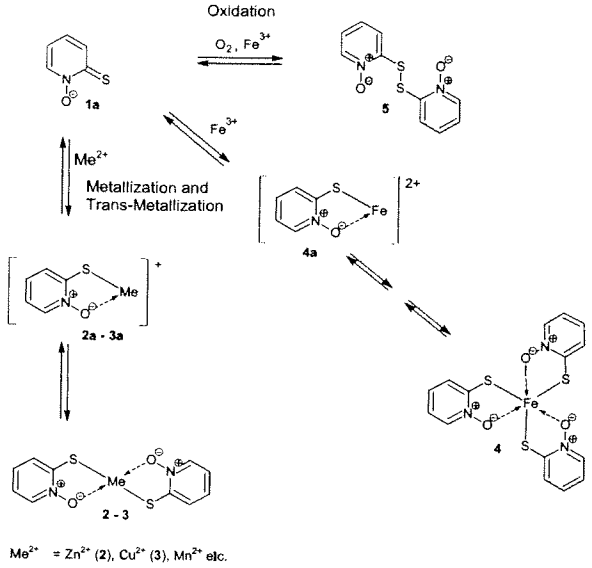


Fig. 7 Abiotic transformation pathways of pyrithiones.

case of the 1 : 1 complex species $ZnPT^+$. $ZnPT_2$, $CuPT_2$ and $FePT_3$ exhibit an increased lipophilic character as compared to the ionic species.

Due to this speciation, different bioavailable pyrithione species might be formed according to the chemical properties of the respective medium (*e.g.*, the metal cation content and pH). In the biological system these species may exert their particular molecular interaction potentials. The neutral species, *i.e.* the free PT, the Cu(II) 1 : 2 or the Fe(III) 1 : 3 complex, could *e.g.* diffuse across the hydrophobic bilayers of cell membranes, immediately dissociating when entering the cytosol. Pyrithione species could coordinate to the active-site metal in metalloenzymes, intercalate in nucleic acids or interfere with electrochemical membrane gradients. It could enter ionic interactions with phospholipid head groups of membrane lipids or disulfide exchange reactions.

Many of these conceivable interactions of pyrithiones with biomolecules have their origin in the N-hydroxythioamide structure of pyrithione. This structure exhibits an inherent neighboring group effect that enables the molecule to chelate Lewis acids of appropriate size and LUMO, and to carry a negative charge. PT possesses a larger number of interaction potentials than would be the sum of interaction potentials of Py, PyNO and PyS.

This explains the toxicity differences of compounds that possess the 2-thiopyridine-1-oxide structure to those without this structure. These results corroborate with findings of Shaw *et al.*,[45] Leonard *et al.*,[46] Albert *et al.*,[2,47] Chandler and Segel[15] and Möller *et al.*,[14] which showed that pyridine derivatives with oxygen bound to the pyridine nitrogen and sulfur (=S/–SH) located adjacent to the nitrogen exhibit far higher biocidal action than structural analogs.

The speciation of the pyrithione metal complexes explains the similarity in toxicity of the metal complexes of pyrithione and NaPT. According to the metal content of the physiological medium different metal complexes of pyrithione could, at low concentrations, afford similar identities and proportions of bioavailable species. However, in the case of $FePT_3$ one would expect a lower effect concentration according to stoichiometry.

Notably, the $(PT)_2$ is as toxic as the pyrithiones although it exhibits the 2-thiopyridine-1-oxide structure in derivatized form thus lacking the negative charge which supports the chelating character. This can be explained by its neutral and hence more lipophilic character compared to the charged pyrithione species. Higher lipophilicity could enhance transport of this species across the hydrophobic membrane bilayers. $(PT)_2$ then could generate PT in the biological medium according to the redox equilibrium $(PT)_2 + 2e^{\ominus} \leftrightarrows 2$ PT (see eqn. 1). This would explain the difference from MSPT, which also possesses the 2-thiopyridine-1-oxide structure in derivatized form but is not in equilibrium with free pyrithione. However, IPC-81 cells may also exhibit molecular structures and regulating processes amenable to interaction or reaction with $(PT)_2$ itself.

5 Conclusion

According to the Green Chemistry principles, chemical products should be designed to effect their desired function while minimizing their toxicity.[48] This of course is challenging in the case of antifouling biocides since their purpose is to prevent fouling by toxic action.

Since the N-hydroxythioamide group appears to be the origin of pyrithione toxicity, care has to be taken that this group breaks down irreversibly in biotic or abiotic pathways. Thus, different environmental conditions need to be considered, in particular with respect to the possible formation of $CuPT_2$, $FePT_3$ and $(PT)_2$ (Fig. 7). These highly toxic compounds might form in environmental media, since oxidants and metal cations like Cu(II) and Fe(III) are ubiquitous. Cupric and ferric ions are present in high concentrations in shipping environments (shipping lines, harbors). Cupric oxide is used as an additive in antifouling paints. The environmental occurrence and fate of $CuPT_2$, $FePT_3$ and $(PT)_2$ need to be investigated and monitored in order to validate sustainability of pyrithione antifoulants that has been recommended by manufacturers.

Manufacturers currently refer to reports of rapid degradation and detoxification of $ZnPT_2$ in synthetic seawater under anaerobic and aerobic conditions.[7,49] Unfortunately, test protocols and the chemical structure of some of the identified transformation products were not provided on request. Other studies showed that microbiological activity of aqueous solutions of NaPT was not affected after storage at 40 °C for three months.[50] Mixtures of cupric carbonate and NaPT, which in long term decay tests were tested for their efficacy as a synergetic wood preservative, exhibited no decay in biological activity for six years.[51]

Investigation of the molecular causalities of the molecular mechanisms of pyrithione action is an attractive research field, and resulting knowledge would aid the decision whether pyrithione antifoulants are sustainable alternatives to tributyltin antifoulants. This requires further investigation on a more mechanistic level beyond the scope of the present study.

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